PATHWAYS OF NUCLEOPOLYHEDROSIS VIRUS INFECTION IN THE GYPSY MOTH, LYMANTRIA DISPAR[1]

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Gypsy moth nucleopolyhedrosis virus polyhedral inclusion bodies dissolve slowly in host digestive fluids, in vitro. Infectious viral material is in the hemocoel two hours after ingestion of inclusion bodies. Hemocytes produce and release nucleocapsids throughout the course of infection, but in the fat body, nearly all nucleocapsids are enveloped and occluded.

Although the virus disease of the gypsy moth was reported as early as 1911 (Reiff 1911), very little attention has been given to the mode of action of the causative agent. This report deals with some of the steps in the pathway of nucleopolyhedrosis virus (NPV) invasion and infection in the gypsy moth.

First of all, we know that gypsy moth larvae can become virus-infected by ingesting polyhedral inclusion bodies (PIBs), but we don't know a great deal about what happens to PIBs once they've been ingested. A recent study of PIB dissolution in gypsy moth digestive fluid indicates that the dissolution process may be rather slow; significant dissolution occurred only after two hours incubation (Shields 1984a). We know that factors such as pH, ionic strength and enzymatic activity can be important in PIB degradation (Nordin and Maddox 1971, Paschke and Summers 1975, Wood 1980), and we know that some conditions, such as starvation, can influence these factors (Kawarabata et al. 1980, Pritchett et al. 1982). But we need to know more about the environment of the gut lumen; about how that environment fluctuates; and especially about those conditions that affect the ability of gypsy moth digestive fluids to dissolve PIBs.

With the dissolution of the PIB matrix protein, enveloped nucleocapsids are released in the gut lumen. Many of the nucleocapsids are carried with the food through the alimentary system and are defecated. But some nucleocapsids apparently find their way into the hemocoel. Exactly how nucleocapsids penetrate the peritrophic membrane and pass through the gut epithelium into the hemocoel has not been determined. However, hemolymph bioassays indicate that infectious viral material is in the hemocoel as early as two hours after ingestion of PIBs (Shields 1984a). Since virus is in the hemocoel so quickly, direct passage of inoculum nucleocapsids from the gut lumen to the hemocoel seems likely. This phenomenon has been reported for Autographa californica NPV in Trichoplusia ni (Granados and Lawler 1981).

For a systemic infection to proceed, once in the hemocoel, nucleocapsids must escape host defense mechanisms, enter susceptible cells, replicate, and progeny nucleocapsids must be released. Of the five types of hemocytes in gypsy moth larvae, three are susceptible to NPV infection - prohemocytes, plasmatocytes and especially coagulocytes. Coagulocytes are the first hemocytes to replicate virus and eventually are selectively depleted from the circulating hemocyte population. Granulocytes and spherulocytes do not seem to be susceptible to NPV.

Nucleocapsids appear to enter gypsy moth hemocytes by two routes. One is by fusion of the viral envelope with the hemocyte plasma membrane, releasing the nucleocapsid into the cytoplasm. Enveloped nucleocapsids also enter hemocytes by viropexis, an engulfment process. The fate of enveloped nucleocapsids that are engulfed is not certain. The vesicles containing the nucleocapsids may fuse with lysosomes and the nucleocapsids may be destroyed (Adams et al. 1977). However, it is possible that the virus might utilize this process for entry and subsequent release into the cytoplasm.

Nonenveloped nucleocapsids in the cytoplasm frequently are in close alignment with microtubules. This association has been observed in other NPV-host systems (Granados 1978, Granados and Lawler 1981) and Granados (1978) has suggested that microtubules might be involved in the movement of nucleocapsids from the cell surface to the nucleus. In some gypsy moth hemocytes, arrays of nucleocapsids and microtubules appear to extend from the cytoplasm into the nucleus (Shields 1984a, 1984b).

Virus morphogenesis in gypsy moth hemocytes is similar to that reported for NPVs in other species (Paschke and Summers 1975, Granados 1980). Nucleocapsids that acquire envelopes within the nucleus are occluded by polymerizing protein, forming PIBs. Increasing numbers of PIBs are seen in hemocytes during the course of the disease, but hemocytes do not produce as many PIBs as do the cells of the fat body (Shields 1984a).

Infection is seen in the fat body much later than in hemocytes, but eventually all of the cells of the fat body seem to be virus-infected. Nearly all of the nucleocapsids produced in fat body cells are enveloped in the nucleus and occluded. Very large numbers of PIBs are produced and few, if any, nucleocapsids are released from fat body cells.

In contrast, hemocytes replicate and release nucleocapsids into the hemocoel throughout the course of the disease. Nonenveloped nucleocapsids bud from hemocyte nuclei in long tubules of nuclear envelope. They may be released into the hemocoel by fusion of the

plasma membrane and outer lamella of the tubules, as was suggested by Nappi and Hammill (1975). Or, they might first be released into the cell cytoplasm through breaks in the tubule lamellae. In any case, many nonenveloped nucleocapsids are in the cytoplasm of infected hemocytes, and many of these nucleocapsids bud through the plasma membrane into the hemocoel. In so doing, they acquire a peplomer-modified envelope derived from the hemocyte plasma membrane. Adams et al. (1977) suggested that enveloped nucleocapsids bearing peplomers are responsible for secondary infection. If so, hemocytes play an important role in intercellular transmission of virus.

Little is known about the effectiveness of gypsy moth defense mechanisms against NPV, or about how changes in host metabolism might affect susceptibility to infection. A recent study shows that gypsy moth larvae, parasitized five days previously by Blepharipa pratensis, are resistant to NPV. Perhaps B. pratensis myiasis activates anti-viral substances in the hemolymph of the host.

More research is needed on all of the factors that might be involved in determining whether NPV will invade amd kill the gypsy moth. Such information is necessary for prediction of viral epizootics and also could be useful in the development of improved viral pesticides.

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[2]Unpublished data, on file, Center for Biological Control of Northeastern Forest Insects & Diseases, Hamden, Connecticut.